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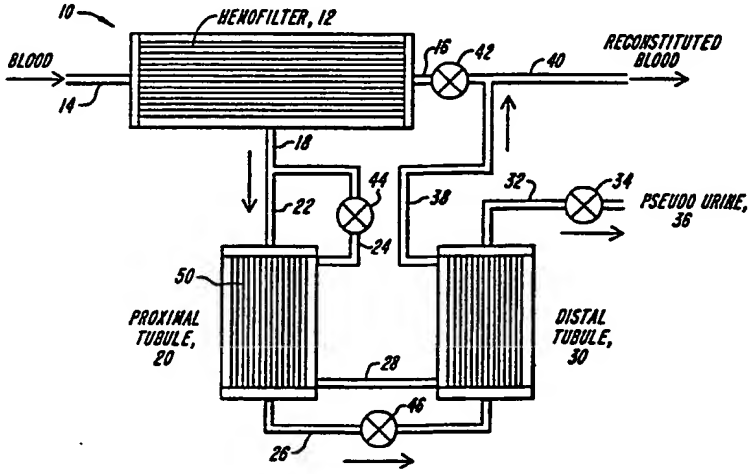

(57) Abstract

An apparatus for processing an aqueous solution, particularly the ultrafiltrate f a hemofiltration unit, (12), is disc sed. The apparatus includes at least one confluent culture of renal epithelial cells grown upon a semipermeable membrane. The solution t be processed is delivered to one side of the membrane, (14), and a processed solution is collected from the ther side of the membrane, (16), and physiological processing by the cells. In the case of blood purification, the processed ultrafiltrate can be recombined with the residue of the hemofilter to reconstitute bl od. The cultured renal epithelial cells provide physiological regulation of organic anion transport across the membrane.

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BLOOD PURIFICATION WITH CULTURED RENAL CELLS

Background of the Invention

The technical field of this invention is blood purification and, in particular, blood filtration systems and methods employing cultured renal cells to provide physiological regulation of solutes in a filtrate.

Healthy human beings excrete about 1.5 liters of urine per day, removing from the body a large number of metabolic waste products and other toxins. The volume of urine excreted, however, is but a small fraction of the fluid processed on a continuous basis by the kidneys. At any given time between 10 and 20 percent of the cardiac output may pass through the kidneys.

The natural renal excretory function is govern by two large-scale opposing processes. In the glomerular capillaries approximately 180 liters of fluid are removed from the blood each day by an ultrafiltration process. Almost all of this fluid (about 99 percent) is reclaimed by the body in the kidney's tubules. The final waste stream is a urine in which the body's metabolic wastes, such as urea, creatinine, uric acid, and electrolytes are highly concentrated.

When renal failure occurs, transplantation or dialysis are available as treatment. Unfortunately, only 35 to 50 percent of patients with end stage renal diseases are suitable for transplantation. For the remainder, peritoneal dialysis or hemodialysis are the only treatment options. Peritoneal dialysis typically involves the instillation of about 2 liters of a dialysate into the peritoneal cavity via a permanent catheter every 4 to 6 hours followed by the removal and replacement of the fluid. It requires a high degree of patient compliance and can result in inadequate clearance, catheter tunnel infection, peritonitis and protein loss.

For most patients (about 80 percent of those on chronic dialysis or awaiting a suitable transplantation donor) hemodialysis is the preferred method of treatment. Hemodialysis involves the diffusion of low molecular weight blood solutes across a semipermeable membrane. A constant stream of the patient's blood flows on one side of the membrane while a dialysate flows on the other side. The dialysate typically is augmented with glucose and various electrolytes, such as sodium, potassium, magnesium, calcium and chloride ions so as to roughly balance the concentration of these solutes on both sides of the membrane.

Because the machinery is very expensive and large quantities of pure water (to prepare the dialysate) are required, almost all hemodialysis patients must rely upon a dialysis center for treatments that typically involve 10 to 15 hours per week, divided into several sessions. Beyond the inconvenience and discomfort, there is a growing realization that dialysis fails to remove many blood solutes of intermediate molecular weight (i.e., metabolic wastes on the order of 300 to 6000 m.w.) that also play a role in metabolic abnormalities. Moreover, the replacement of blood electrolytes via the dialysate solution is often less than satisfactory because the technique does not take into account the amount of electrolytes needed by individual patients.

A related approach to blood cleansing in chronic renal failure is hemofiltration, whereby the blood is filtered through a selectively permeable membrane to remove water and low as well as middle sized molecular solutes (e.g., up to 50,000 m.w.). In such systems, large amounts of replacement fluids must be added either before or after the ultrafiltration device to reconstitute the blood. To avoid the need for large quantities of sterile replacement fluids, sorbent regeneration (or dialysis) followed by reinfusion of the ultrafiltrate has been attempted. Even with reinfusion of a purified ultrafiltrate, however, the problem of maintaining the proper electrolyte balance for individual patients remains unsolved.

There exists a need for better techniques and devices for processing the ultrafiltrate while performing hemofiltration in order to restore the necessary fluids and solutes to the blood. More generally, there also exists a need for blood purification techniques that remove waste products in a fashion similar to the natural physiological excretion processes. In particular, methods and apparatus that could address the electrolyte balancing problems of renal dysfunction on an individualized basis would satisfy a long felt need in the field.

Summary of the Invention

It has been discovered that renal epithelial cells can be grown on semipermeable membranes, such as hollow polymeric fibers, to provide an approximation of the renal nephrons. The invention permits physiological-type regulation of ion and molecular transport, especially on the concentration of urine from an ultrafiltrate. The invention is particularly useful in hemofiltration to process ultrafiltrate and provide a sterile fluid suitable for blood reconstitution. A bioartificial kidney is disclosed in which cultured renal cells are employed to respond to fluctuations in the patient's electrolyte levels and to increase the elimination of certain waste products through active secretory mechanisms.

In one aspect of the invention, an apparatus for processing an aqueous solution, particularly the ultrafiltrate of a hemofiltration unit, is disclosed. The apparatus includes at least one confluent culture of renal epithelial cells grown upon a semipermeable membrane. The solution to be processed is delivered to one side of the membrane and a processed solution is collected from the other side of the membrane after physiological processing by the cells. In the case of blood purification, the processed ultrafiltrate can be recombined with the residue of the hemofilter to reconstitute blood. The cultured renal epithelial cells provide physiological regulation of organic anion transport across the membrane.

In another aspect of the invention, two or more serial processing units can be employed to approximate the functions of the proximal and distal segments of the natural nephron. Thus, cells derived from a proximal nephron segment (e.g., the proximal tubule) can be employed in a first processing element and cell derived from a distal segment (e.g., the distal tubule or the cortical collecting tubule) can be employed in a second (or further) processing element. The cells are preferably plated onto the membranes prior to polarization.

The processing units of the invention include renal epithelial cells of various types grown on semipermeable membranes. The membranes allow the exchange of various ions and other ultrafiltrate components under the physiological control of the renal cells. Preferably, the membranes in the ultrafiltrate processing units allow the passage therethrough of molecules having molecular weights up to about 1,000,000, more preferably, the semipermeable membranes should have an upper limit of permeability in the range of about 30,000 to about 200,000 daltons. The membranes can vary in thickness from about 5 microns to about 100 microns.

The membranes are preferably tubular membranes with the renal cells grown on the inside. The optimal membrane will depend on the cell line. Acrylic copolymer, polysulfone, polyacrylonitrile, nitrocellulose and cellulose acetate membranes can be used in particular applications. Various attachment factors, such as collagen and extracellular matrices can be used to induce cell attachment to the membranes.

The invention will next be described in connection with certain illustrated embodiments; however, it should be clear that various modifications, additions and subtractions can be made without departing from the spirit or the scope of the invention. For example, various commercially available hemofiltration devices can be employed to obtain an ultrafiltrate. The hemofilter typically includes a matrix of flat or hollow fiber membranes which filters water and low molecular weight solutes from the blood. Preferably, the hemofilter should have an upper limit of permeability in the range of about 30,000 to about 50,000 molecular weight to exclude viruses, antibodies, antibodies and complement.

The apparatus can be employed in extracorporeal devices or be worn by the patient. It may also be implantable, and the waste liquid can be drained into the urinary bladder. The apparatus can rely on the patient's own heart for pumping or can employ an auxiliary pumping element. Moreover, additional valves, sensors and monitors as known to those skilled in the art can be incorporated to further regulate the operations of the apparatus.

Brief Description of the Drawings

FIG. 1 is a schematic illustration of a blood purification device according to the present inventor.

FIG. 2 is a schematic cross-sectional illustration of a tubular membrane and a confluent layer of renal epithelial cells grown on the inner surface thereof, for use in the device of FIG. 1.

Detailed Description

In FIG. 1, a schematic blood purification system 10 according to the present invention is shown, including hemofilter 12, and a proximal tubule 20 and a distal tubule processing unit 30. The tubule units contain cultured renal cells adapted to serially process an ultrafiltrate from the hemofilter 12.

The hemofilter 12 includes an inlet 14 adapted to receive a patient's blood and an outlet 16 from which the residual blood exits. The filter is typically composed of a very large number of parallel, hollow ultrafiltration fibers. During filtration, water and low molecular weight solutes are drawn from the blood as an ultrafiltrate via port 18. The fibers in hemofilter 12 define membranes which provide an immunoprotection barrier separating the cultured cells of the processing units 20 and 30 from viruses, bacteria, antibodies and complement which may be present in the patient's blood.

The proximal tubule processing unit 20 includes an inlet 22 to receive the ultrafiltrate from hemofilter 12 and an outlet 26. Within the proximal tubule processing unit 20, the ultrafiltrate flows through parallel hollow fibers. Inside the fibers, renal epithelial cells which have been grown to confluence establish an electrochemical gradient.

A portion of the ultrafiltrate is supplied to the outside of fibers via shunt 24 to provide a medium for secretion and to supply hormones to the baso-lateral side of the cultured cells. The cells cultured in the proximal tubule processing unit 20 of the invention are preferably obtained from an animal or human cell line which originated in the proximal tubule of the donor in order to approximate the functions of the proximal tubule in a natural kidney (e.g., sodium dependent glucose uptake).

The processed ultrafiltrate from the proximal tubule 20 processing unit is delivered to the inlet port 26 of the distal tubule processing unit 30. At the same time, the ultrafiltrate stream which was circulated on the outside of the fibers of the proximal unit is delivered via shunt 28 to the outside of the distal unit's fibers.

The distal tubule processing unit 30 similarly contains parallel fibers, inside of which renal cells have been grown to confluence. The cells in the distal unit 30 are likewise chosen to approximate the functions of the distal tubule in a natural kidney. The output 32 of the distal unit 30 is discharged as a pseudo urine 36. The discharge can be controlled by flow rate control valve 34. The remaining ultrafiltrate 38 which has been physiologically processed by the cultured cells of the tubules 20 and 30 is mixed with filtered blood 16

from the hemofilter 12 to provide the patient with reconstituted blood 40. Values 42, 44 and 46 can also be employed to control the flow rates at various stages of the system, as shown. The valves can be automatically adjustable in response to processing conditions or can be preset based upon experience or established protocols.

FIG. 2 is a schematic illustration of an individual processing element 50 from a tubule unit, including a hollow semipermeable fiber 52 and an internal confluent culture of renal epithelial cells 54. The fiber 52 is preferably a polyvinyl chloride acrylic copolymer (XM-60; 0.3 mm OD; 0.2 mm ID; Amicon Corp., Lexington, MA) or a polysulfone (F-60; 0.3 mm OD; 0.2 mm ID; Fresenius Corp., West Germany). The cells are grown to form a confluent culture of tight junctions.

To further illustrate the present invention, a series of experiments were conducted to demonstrate the feasibility of culturing renal cells within hollow fibers and the ability of such cells to provide physiological processing of aqueous solutions.

Renal epithelial cell lines (MDCK and LLC-PK_1) were obtained from American Type Culture Collection (ATTC, Rockville, MD). Stock cultures were maintained at 37°C in a humidified incubator in a 5% CO_2/95% air mixture on tissue culture grade polystyrene 60 mm Petri dishes (Lux Contur Permanox, Miles Scientific, Naperbill, IL) with Dulbecco's Modified Eagle's medium (DMEM; Gibco, Grand Island

Biological Co., Grand Island, NU) containing 10% fetal calf serum (FCS; Gibco), 100 U/ml penicillin, 100 ny/ml streptomycin (Sigma, St. Louis, MO), and 2.5 µg/ml of amphotericin B. The culture medium was changed three times per week. The cells were subcultured by rinsing the dish twice with Ca^{++} and Mg^{++} free Hanks Balanced Salt Solution HHBSS, Gioco) before incubating the monolayer with 0.25 ml of 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) at 37°C for 20 to 30 min with occasional stirring. The dissociated cells were washed once in DMEM-FCS before being used for passage or for seeding the prepared membranes.

Polyvinyl chloride acrylic copolymer (XM-50; 0.3 mm OD, 0.2 mm ID; Amicon Corp., Lexington, MA), and polysulfone (PS) hollow fibers (F-60; 0.3 mm OD, 0.2 mm ID; Fresenius Corp., West Germany), 3.0 cm in length, were grouped in bundles of 20 and secured with 3-0 silk ligatures. The PS fibers were boiled in physiologic saline to remove any residual chemicals before further processing. All bundles were washed in 0.9% saline for removal of particulate debris, 50% ethanol to wet the material, 0.1 N HCl to sterilize the material, and, finally, sterile phosphate buffered saline. Depending upon the particular experiment, the bundles were then dipped in either a 1:4 diluted solution of collagen composed of 95% Type I, 5% Type III (Vitrogen, Collagen Corp., Palo Alto, CA), or extracellular matrix (Matrigel, Collaborative Research, Waltham MA), which was allowed to polymerize overnight at 37°C. The bundles were then soaked in a Petri dish containing culture

medium for 2 to 4 h before seeding with cells at a density of 1 x 10^6 MDCK or LLC-PK_1 cells per bundle. For culturing we used either DMEM, as described above, or a serum free hormonally defined medium (HDM) made of a 1:1 mixture of Ham's F12 nutrient mixture (Gibco) and DMEM, to which selenium, transferring, insulin, hydrocortisone, and triiodothyronine were added. Additionally, prostaglandin E_1 was added to the MDCK cell culture media, and cholesterol and vasopressin were added to the LLC-PK_1 cell culture media. The media were changed three times per week.

MDCK and LLC-PK_1 cells were also cultivated in HDM on Millicell-HA membranes (Millipore, Bedford, MA), which are microporous nitrocellulose membranes mounted on a polystyrene cylinder with three small feet at the bottom. When placed in a Petri dish, the membrane separates two liquid compartments. In some experiments, the nitrocellulose membrane was replaced by an impermeable polystyrene disk.

At defined time intervals, bundles were taken out of their Petri dishes and gently washed with Ca^{++} and Mg^{++} free HBSS. They were then immersed in a solution of 0.25% trypsin-EDTA for 60 to 120 min at 37°C to detach the cells, which were then counted with the use of a hemocytometer.

Bundles fixed in 4% paraformaldehyde / 1% glutaldehyde in 0.1 M cacodylate buffer (pH 7.5) were then postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.5, washed, dehydrated, critical point dried, sputter coated with gold-palladium, and examined under a scanning electron microscope (AMRay 1,000A).

Bundles fixed in 4% paraformaldehyde / 1% gluteraldehyde in 0.1 M cacodylate buffer, pH 7.5, were then postfixed with a 1% osmium tetroxide solution, washed in buffer, dehydrated, and embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate for examination under a Phillips 410 transmission electron microscope.

Transepithelial electrical resistance was used to recognize the development of a confluent, tight cell layer on the cell-seeded Millicells.

Measurements of electrical resistance were performed with a Petri dish cover modified to hold 4 Ag/AgCl electrodes. Two electrodes contacted to the solution in the apical compartment, whereas the other two made contact in the basal compartment. These electrodes were connected to an automatic voltage-clamp amplifier (DVC-1000) Voltage/Current Clamp, World Precision Instrument, New Haen, CT). The potential difference between the apical and basal compartment (electrodes 1 and 2) was preset at given values (-30 to 30 mV), and the voltage-clamp amplifier automatically regulated current pulses (through electrodes 3 and 4) to maintain the desired

voltage difference with a typical clamp time of about 4 s. Sets of voltage/current values were recorded at room temperature in a laminar flow hood at regular time intervals during cell growth. The total electrical resistance across the cell layer (ohm-cm^2) was calculated as the slope of the linear regression of the current versus voltage values. Some cell covered membranes were fixed and processed for light microscopic examination to correlate cell proliferation and electrical resistance.

To assess the ability of a confluent monolayer of kidney epithelial cells grown on a permeable synthetic membrane to transport selective solute, the concentration of phenol red was measured in both basal and apical liquid compartments before and after confluence in cell seeded Millicell devices. The same measurements were performed in unseeded Millicells and Millicells whose permeable membranes had been replaced by an impermeable polystyrene disk. At time zero, fresh HDM containing 15 mg/l of phenol red was placed in both liquid compartments. Phenol red concentration was determined by measuring the concentration of phenol red in 500 µl samples through absorbance at 560 nm after alkalinization with NaOH with the use of a DU-65 spectrophotometer (Beckman, Fullerton, CA).

The Wilcoxon-Mann-Whitney test (rank sum test) served to assess statistical differences ($P<0.05$) between various populations. All results are presented as mean plus or minus standard deviation.

MDCK cells grown on acrylic copolymer (AC) hollow fibers reached confluence in less than 3 weeks. The cells formed a flat monolayer. They exhibited polarized morphologic characteristics, including short apical microvilli, tight junctions, desmosomes, and some basolateral infolding and close attachment to the membrane. Polarized morphologic characteristics were recognizable in all cells, including those located on the inferior surface of the hollow fibers. A thin basement membrane separated the cell layer and the polymer surface. Longer microvilli were observed when the cells were cultured in HDM. There was no recognizable morphologic difference between precoated and uncoated AC fibers.

On PS fibers, the apical microvilli of MDCK cells grown in serum supplemented medium were dense, and their cell borders were well defined, but they had not reached confluence at 5 weeks. Some degree of multilayering was observed, especially at the growing edge. Pseudopodic cytoplasmic extension often penetrated into the pores of the PS membrane. Microvilli were also present on the lateral surfaces of the cells. Cells grown in HDM also displayed longer microvilli than those grown in serum. supplemented medium, but there was no morphologic difference between precoated and uncoated fibers.

The LLC-PK_1 cell line showed the same general morphologic characteristics, although the differences between polymer substrates seemed less pronounced than with the MDCK cells.

With the exception of day 3, the number of MDCK cells was significantly greater when they were cultivated with serum supplemented medium as compared with HDM. This applied to both uncoated AC and PS hollow fibers and was observed whether the fibers were coated with collagen or with extracellular matrix. When comparing uncoated polymers, the number of cells was significantly greater on the AC fibers than on the PS fibers in the serum supplemented medium, whereas comparable numbers were observed in the HDM.

Either collagen or extracellular matrix precoated hollow fibers allowed growth of significantly greater cell numbers for both polymeric substrates and culture media compared with uncoated hollow fibers, with the exception of AC fibers placed in serum supplemented medium. Although not consistent for all time periods, hollow fibers coated with collagen appeared to show more extensive cell coverage than the fibers coated extracellular matrix.

Kidney epithelial cells seeded on the nitrocellulose membrane of the Millicell devices proliferated in a patchy pattern. Once the patches became confluent, the transmembrane electrical resistance began to increase from a value around 50 ohm-cm^2 for the unseeded membranes and the seeded membranes during the first week to values ranging from 150 to 20 ohm-cm^2 during the second week, These values were maintained through the third week until the experiment was terminated.

There was no difference in phenol red concentration between the apical and basal compartments of cell seeded Millicell devices until confluence was established. From that point on, a significant continuous increase in the ratio of apical to basal concentration of phenol red was observed during the first 36 h, reaching an apical/basal ratio of 1.2 ± 0.05 after 36 h. This concentration difference was then maintained up to 60 h when the experiment was terminated. In contrast, no increase of phenol red concentration was observed with unseeded Millicells. A slight decrease of the apical/basal phenol red ratio was noticed in control experiments where the permeable membrane of the Millicell device had been replaced by an impermeable polystyrene disk.

The results show that feasibility of culturing kidney epithelial cells to confluence on permselective hollow fibers. Several factors seem to influence the morphologic features of these cells on permselective membranes. The density and length of the apical microvilli are influenced by the polymer substrate and the culture medium used. Extensive basolateral infolding, well defined cell borders, and some degree of multilayering are observed with MDCK cells grown on PS membranes. Multilayering may result from the surface geometry of the PS membrane, which allows extension of cellular processes into its

pores, increasing cell anchorage and making lateral cellular proliferation more difficult. Longer microvilli are observed on cells cultured in a HDM as compared with a serum supplemented medium, suggesting more advanced differentiation. It is generally believed that the absence of various inhibitory serum factors allows the cells to better differentiate.

With the use of morphological criteria, no difference was noticed between uncoated and precoated fibers nor between fibers coated with collagen and those coated with extracellular matrix, suggesting that the polymer substrate and the medium used may be more important than coating for the differentiation of the renal cells. However, collagen and extracellular matrix noticeably improved cell proliferation on both types of membrane with either type of medium.

Cell proliferation was more pronounced in serum supplemented medium than in HDM. With the serum supplemented medium, no beneficial effect of coating was observed on AC fibers, perhaps because the highly hydrophilic AC membrane may have favored absorption of serum proteins and made the addition of attachment factors such as collagen or extracellular matrix superfluous, whereas the less hydrophilic PS still requires those additional attachment factors for cell attachment and growth. These results suggest that plasma proteins, collagen, and extracellular matrix are important for the anchorage and proliferation of the renal epithelial cells on a polymer substrate, whereas hormones and the absence of inhibitory serum factors may be influential for the expression of differentiation.

This formation of tight, confluent monolayer of renal epithelial cells is a prerequisite for the establishment of transepithelial electrochemical gradients and selective solute transport. With careful sterile techniques, an increase in transmembrane electrical resistance appeared to be a reliable indicator for cell confluence. Once confluence was reached, an apical/basal gradient of phenol red was observed, whereas no gradient was present when the membrane was not seeded with cells or if the cells had not yet reached confluence. The use of an impermeable membrane slightly inverted the gradient. This may be explained by a higher surface area to volume ratio in the basal compartment than in the apical compartment, allowing evaporation, which inevitably occurs even in a humidified incubator, to concentrate phenol red in the basal compared with a greater extent than in the apical compartment.

The observation of an apical/basal gradient of phenol red with permeable membranes covered with kidney epithelial cells therefore demonstrates active solute transport. The ability of a bioartificial kidney to secrete organic anions may have clinical relevance because organic anions have been incriminated as toxins in the uremic syndrome.

Because the permselective membranes of the hemofilter can provide immunoprotection to the cultured cells, there should be no restrictions on the animal species used in construction bioartificial kidneys in accordance with this invention. Moreover,

the present invention can provide immunogenic isolation of the cultured renal cells from the patient, especially when the cells are cultured on the inside of the membrane (e.g., exposed only to the fluid which is discharged as pseudo urine).

These studies demonstrate that 1) renal epithelial cells can be cultured to confluence in a polarized manner on semipermeable hollow fibers; 2) the polymeric substrate and the culture medium influence the degree of differentiation of the cells, whereas cell attachment factors favor their proliferation; 3) the establishment of a transmembrane resistance indicates that a renal epithelial monolayer can become electrically tight when grown on a permeable polymeric substrate, an essential prerequisite for the establishment of an electrochemical gradient; and 4) an electrically tight monolayer of kidney epithelial cells on a permeable membrane displays active transport properties such as phenol red secretion.

Thus, an exchanger, whose permeable membranes are covered by functional kidney epithelial cells, can allow preferential solute transport and, when combined with a continuous ultrafiltration device, can transform an ultrafiltrate into a urine.

Claims

1. An apparatus for processing an aqueous solution, the apparatus comprising a confluent culture of renal epithelial cells grown upon a semipermeable membrane, means for delivering an aqueous solution to one side of the membrane and means for removing a processed solution from the other side of the membrane.

2. The apparatus of claim 1 wherein the membrane is an acrylic copolymer membrane.

3. The apparatus of claim 1 wherein the the membrane is a polysulfone membrane.

4. The apparatus of claim 1 wherein the membrane is a nitrocellulose membrane.

5. The apparatus of claim 1 wherein the membrane is a polyacrylonitrile membrane.

6. The apparatus of claim 1 wherein the membrane is a hollow fiber, tubular membrane.

7. The apparatus of claim 1 wherein the membrane is a hollow fiber, tubular membrane and the cells are cultured on the inside of said tubular membrane.

8. The apparatus of claim 1 wherein the cells are derived from a proximal nephron segment.

9. The apparatus of claim 1 wherein the cells are derived from a distal nephron segment.

10. The apparatus of claim 1 wherein th aqueous solution is an ultrafiltrate from a hemofilter.

11. The apparatus of claim 1 wherein the aqueous solution is an ultrafiltrate from a hemofilter and the apparatus further includes means for adding the processed solution to the residue of the hemofilter to reconstitute a blood product.

12. The apparatus of claim 1 wherein the apparatus further comprises an immunoprotection barrier which excludes viruses, bacteria, antibodies and complement from the aqueous solution prior to processing.

13. A method of processing an aqueous solution, the method comprising:

growing a culture of renal epithelial cells to confluence on a semipermeable membrane;

delivering an aqueous solution to one side of said membrane; and

collecting a processed solution from the other side of the membrane.

14. The method of claim 13 wherein the step of growing the cells further includes applying an attachment factor to the membrane prior to seeding the cells on the membrane.

15. The method of claim 14 wherein the method further includes applying collagen as an attachment factor.

16. The method of claim 14 wherein the method further includes applying an extracellular matrix as an attachment factor.

17. The method of claim 13 wherein the step of growing the cells further includes growing the cells in a CO_2 controlled incubator.

18. The method of claim 13 wherein the step of growing the cells further includes growing the cells in a serum-supplemented medium.

19. The method of claim 13 wherein the step of growing the cells further includes growing the cells in a hormonally defined medium.

20. The method of claim 13 wherein the step of growing the cells further includes growing cells derived from a proximal nephron segment.

21. The method of claim 13 wherein the step of growing the cells further includes growing cells derived from a distal nephron segment.

22. The method of claim 13 wherein the step of delivering an aqueous solution to one side of the membrane further includes delivering an ultrafiltrate from a hemofilter to the membrane.

23. The method of claim 13 wherein the step of delivering an aqueous solution further includes delivering a solution substantially free of viruses, bacteria, antibodies and complement.

24. An apparatus for removing substances from blood, the apparatus comprising:

a hemofilter capable of separating blood into an ultrafiltrate of water and low molecular weight solutes, and a residue of blood cells and high molecular weight blood components;

at least one ultrafiltrate processor comprising a culture of confluent renal epithelial cells grown upon a semipermeable membrane, means for delivering the ultrafiltrate to one side of the membrane and means for collecting a processed ultrafiltrate from the other side of the membrane whereby unwanted substances are removed from said ultrafiltrate; and

means for combining said hemofilter residue and said processed ultrafiltrate to reconstitute blood.

25. The apparatus of claim 24 wherein the membrane is an acrylic copolymer membrane.

26. The apparatus of claim 24 wherein the the membrane is a polysulfone membrane.

27. The apparatus of claim 24 wherein the membrane is a nitrocellulose membrane.

28. The apparatus of claim 24 wherein the membrane is a polyacrylonitrile membrane.

29. The apparatus of claim 24 wherein the membrane is a hollow fiber, tubular membrane.

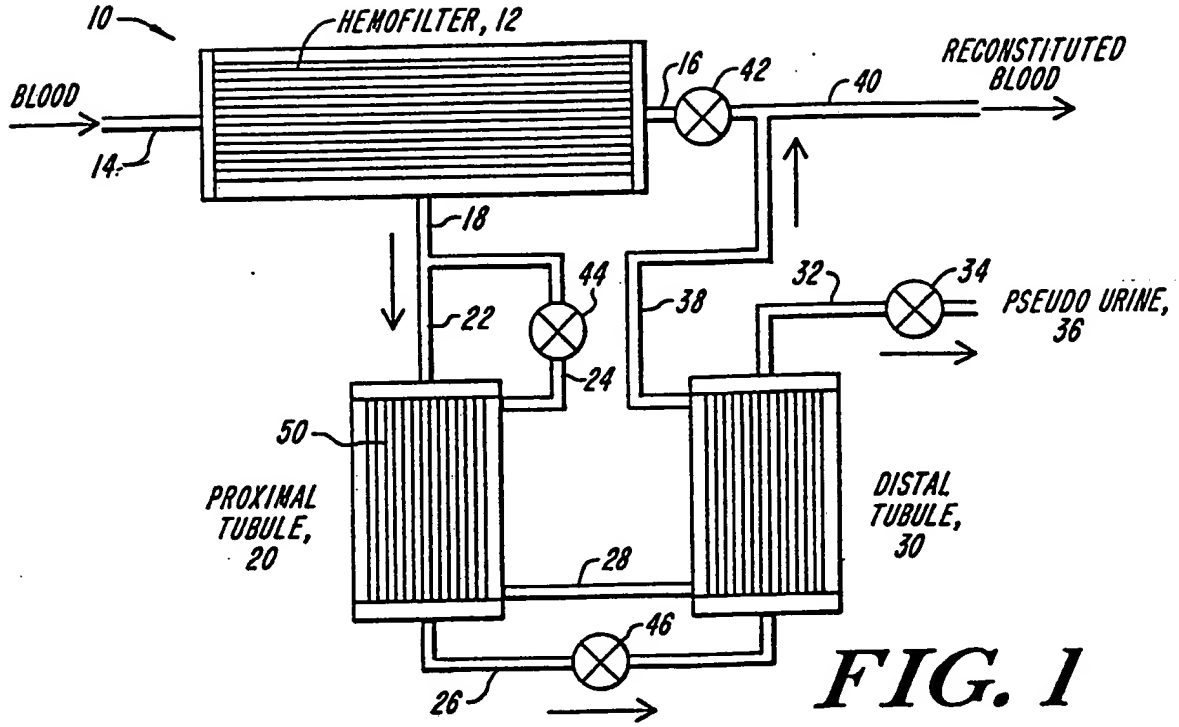
30. The apparatus of claim 24 wherein the membrane is a hollow fiber, tubular membrane and the cells and cultured on the inside of said tubular membrane.

31. The apparatus of claim 24 wherein the cells are derived from a proximal nephron segment.

32. The apparatus of claim 24 wherein the cells are derived from a distal nephron segment.

33. The apparatus of claim 24 wherein the ultrafiltrate processor further comprises a first membrane on which cells derived from a proximal nephron segment are grown, and a second membrane on which cells from a distal nephron segment are grown.

34. The apparatus of claim 24 wherein the hemofilter further includes an immunoprotection barrier which excludes viruses, bacteria, antibodies and complement.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BLOOD
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RECONSTITUTED BLOOD
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PSEUDO URINE, 36
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PROXIMAL TUBULE, 20
DISTAL TUBULE, 30
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FIG. 1

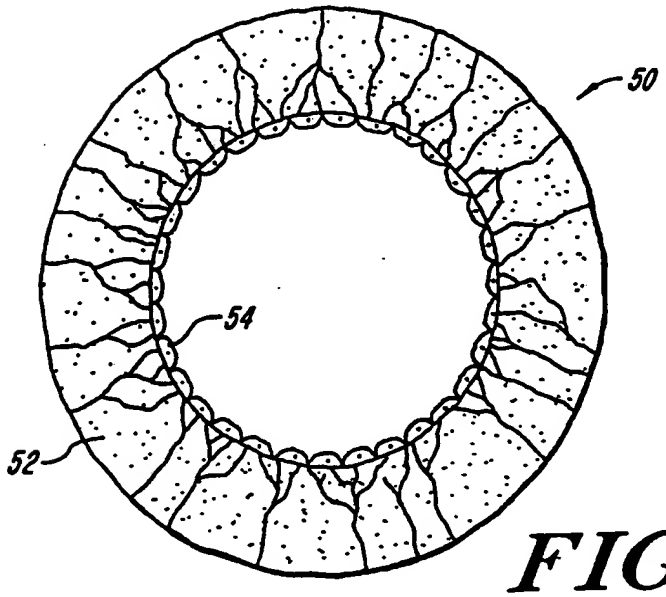
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FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/02919

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4) C12N 05/00 A01N 01/02 C12M 01/12

US Cl 435/(240.241) 435/(283) 435/(311)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US:	435/240.241 435/283 435/311

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Computer searched CAS 1967-1988, Biosis 1969-1988, APS 1975-1988.

See Attachment on search terms.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US,A3579441 (Brown) 18 May 1971. See Whole Document.	1-34
Y	US,A4610791 (Henne et al) 09 September 1986. See Whole Document.	1-34
Y	US,A4087327 (Federetal) 02 May 1978. See Whole Document.	1-34
Y	Culture of Animal Cells, 1983. Freshney. Alan R. Liss Publishers, NY page 56.	14-16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21 October 1988	25 JAN 1989
International Searching Authority	Signature of Authorized Officer
ISA/US	Lori F. Beadell

Attachment to PCT/ISA/210
Part II Field Search

Search Terms

1. hollow fiber membrane
2. acrylic, polysulfone, nitrocellulose, polyacrylonitrile or cellulose acetate
3. renal epithelia
4. nephron or kidney
5. filter or hemofilter
6. virus, bacteria or antibody
7. blood serum or plasma